

The Neuroendocrine Response of Luteinizing Hormone to Estrogen Administration in Heterosexual, Homosexual, and Transsexual Subjects

LOUIS GOOREN

Division of Endocrinology, Hospital of the Vrije Universiteit, 1007 MB Amsterdam, The Netherlands

ABSTRACT. The neuroendocrine response of LH to estrogen administration may be related to sexual dimorphism of the brain, and therefore, homosexual and especially transsexual individuals may differ from heterosexual individuals in their responses. This study failed to find such differences among groups of female heterosexuals, homosexuals, and transsexuals. Specifically, after single dose estrogen administration, all subjects had an initial decline in serum LH levels, followed by a brisk rise of equal magnitude. Among males, the type of response was less uniform. After an initial fall in serum LH levels, the individual responses varied. In 12 of 23 male homosexuals, 10 of 15 male heterosexuals, and all 6 genetic male transsexuals studied, serum LH levels remained below pretreatment levels. In the remaining 11 male homosexuals and 5 of the heterosexuals, serum LH levels increased to values exceeding those before treatment, resembling

the response found in the 3 groups of women. Those homosexual and heterosexual men with a rise in serum LH levels to above pretreatment values also had the greatest fall in testosterone levels after estrogen administration, while these same men had the lowest testosterone response to hCG stimulation. I conclude from these results that 1) the similarity of LH responses to estrogen administration in all groups of women studied does not support a theory of brain androgenization as a factor in the establishment of gender identity of sexual orientation; and 2) individual differences in men in the type of LH response to estrogen administration can be satisfactorily explained by endocrine factors, such as Leydig cell function, and need not be related to gender identity, sexual orientation, or other possible causes. (*J Clin Endocrinol Metab* 63: 583, 1986)

THE NEUROENDOCRINE response of LH to estrogen administration has been regarded as an expression of the sexual dimorphism of the central nervous system, which itself has been linked to such phenomena as sexual orientation and gender identity (1, 2). Reports that a single dose of conjugated estrogens could serve as a possible marker of sexual orientation or gender identity prompted testing this hypothesis in groups of male and female transsexual, homosexual, and heterosexual subjects (1, 2).

The nature of the estrogen response in lower mammals (rats, mice, and guinea pigs) is truly sex specific. Adult female animals receiving an administration of estrogen of sufficient dosage and duration of action at a certain point in their estrous cycle respond with an initial decline in LH levels, followed by a subsequent rise (3, 4). This leads to a greater LH response to LHRH stimulation (5, 6). The capacity for this response appears to be abolished in the male neuroendocrine system, provided it has undergone exposure of sufficient magnitude and duration

to androgens at a critical period in fetal and/or perinatal development (1, 3, 4, 7), a process that has been termed brain androgenization.

Whether the absolute nature of the estrogen response also applies in humans and other higher mammals is less certain. An impaired response in transsexual genetic females was reported (8), but was not confirmed by other studies (9, 10). Recent evidence also indicates that the pattern of estrogen response typically found in females can also be evoked in the male rhesus monkey (11) and the human genetic male (12-17), provided that an estrogen stimulus of sufficient intensity and duration is administered.

Subjects and Methods

Subjects

The transsexual subjects for this study, selected from our clients, included six genetic female transsexuals, aged 27-32 yr, accepted for male role reassignment and six genetic male transsexuals, aged 21-27 yr, accepted for female role reassignment. None of these subjects had previously taken hormonal preparations, and physical examination revealed no abnormalities in any of them. All of the genetic female transsexuals had regular cycles of normal length, and all produced two recent biphasic basal temperature curve charts.

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Address all correspondence and requests for reprints to: Dr. L. Gooren, Department of Internal Medicine, Academisch Ziekenhuis Vrije Universiteit, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands.

Six homosexual women, aged 24–32 yr, all professing a life-long history of exclusive or near-exclusive homosexual orientation, and six heterosexual women, aged 22–27 yr, were studied. None had used hormonal preparations of any kind, including hormonal contraceptives, or any other drugs for at least 15 months before the study.

Twenty-three homosexual men, aged 24–42 yr, were recruited after screening for drug use and medical or endocrine abnormalities, which precluded participation in the study. All proclaimed life-long exclusively homosexual orientation. The 15 heterosexual men, aged 24–39 yr, were all married and reported exclusively heterosexual orientation. Physical examination was normal in all of the subjects.

Serum levels of LH, testosterone, and estradiol were determined in all subjects (in the women between days 3–6 of the cycle) before admission to the experiment. In all subjects, these were within the appropriate reference values of our laboratory.

Study protocol

All subjects gave their informed consent to participation in the study, which was approved by the hospital ethical committee.

Female subjects commenced the experiment between days 3–5 of their cycles; males began at random. Male homosexuals and transsexuals received 50 μ g LHRH, iv, and blood for LH determination was collected 0, 20, 40, and 60 min thereafter. The same test was carried out on the female transsexuals and, for comparative purposes, on the heterosexual females.

On the following day, a single iv injection of 20 mg conjugated estrogens (Premarin, Ayerst, Rouses Point, NY) was given between 0830 and 1000 h. Blood was sampled on the following 4 days between 0830 and 1000 h. After sampling on the fourth day, 50 μ g LHRH were again administered iv to those subjects who had undergone LHRH testing before the administration of conjugated estrogens. LH levels were measured at 0, 20, 40, and 60 min to test for an increased LH response to LHRH stimulation after estrogen exposure, as normally occurs in women (5, 6). In the men, serum testosterone levels were measured before and during the 4 days following administration of conjugated estrogens. In five heterosexual and six homosexual men who had a pronounced LH response to conjugated estrogens (defined as serum LH levels 4 days after administration of conjugated estrogens higher than those on days -1 and 0), the testosterone response to hCG administration was measured and compared to that of six other heterosexual and seven other homosexual men of their groups in whom serum LH levels on days after administration of conjugated estrogens remained below pretreatment values. Serum testosterone levels were measured between 0830 and 1030 h before a single dose of 1500 IU hCG and 24, 48, and 72 h thereafter. A minimum of 5 and a maximum of 9 weeks intervened between measurements of the LH response to conjugated estrogens and the testosterone response to hCG.

Blood was centrifuged immediately after sampling at 4 C and stored at -20 C until assayed. All samples from an individual subject were analyzed in a single assay run. Serum LH was determined in duplicate by a double antibody solid phase RIA using a commercial kit (IRE, Fleurus, Belgium), with a standard

calibrated with the MRC 68/40 preparation. The inter- and intra-assay coefficients of variation were 11% and 7%, respectively. Reference values of serum LH for normal men were 3.0–8.0 mIU/ml; for women in the early follicular phase of the cycle, they were 2.5–6.0 mIU/ml. Serum testosterone was measured in duplicate by RIA using a 7 α -conjugated estrogen-thioether testosterone antiserum. Intra- and interassay coefficients of variation were 9.1% and 12.5%, respectively. Reference values for normal men were 2.30–6.90 ng/ml, and those for women were 0.15–0.50 ng/ml. Serum 17 β -estradiol was determined by Bioscientia (Ingelheim, Germany). After extraction of estrogens from serum and purification and chromatographic separation from estrone and estriol, 17 β -estradiol was quantitated by RIA. The intra- and interassay coefficients of variation were 7.4% and 12%, respectively. Reference values for normal men were 10.9–32.0 pg/ml, and those for women (early follicular phase) were 20–45 pg/ml.

Analysis of the data was done by analysis of variance, split plot pq design (18). The results are presented as the mean \pm SD.

Results

No differences in basal serum LH, testosterone, and estradiol levels were found among the three groups of genetic females. Mean serum LH was 5.0 \pm 1.0 mIU/ml in the heterosexuals, 5.2 \pm 1.2 in the homosexuals, and 4.9 \pm 1.1 in the transsexuals. Basal serum estradiol levels were 34.0 \pm 10.0 pg/ml in the heterosexuals, 36.0 \pm 9.0 in the homosexuals, and 32.0 \pm 11.0 in the transsexuals. Basal serum testosterone levels were 0.32 \pm 0.10 ng/ml in the heterosexuals, 0.29 \pm 0.11 in the homosexuals, and 0.34 \pm 0.13 in the transsexuals. No differences were found in the response of serum LH levels to the administration of conjugated estrogens in the three groups of females (Fig. 1).

An increased serum LH response to LHRH stimulation after administration of conjugated estrogens was found in the female transsexuals (Fig. 2). This was of the same magnitude as in the heterosexual females both before and after administration of conjugated estrogens (Fig. 2).

In the corresponding three groups of genetic males no differences in basal serum LH, testosterone, and estradiol levels were found. Mean basal serum LH was 5.2 \pm 1.3 mIU/ml in the heterosexuals, 5.2 \pm 1.1 in the homosexuals, and 4.8 \pm 1.2 in the transsexuals. Basal serum testosterone was 5.70 \pm 0.60 ng/ml in the heterosexuals, 5.80 \pm 0.79 in the homosexuals, and 6.20 \pm 0.50 in the transsexuals. Basal serum estradiol was 20.5 \pm 6.0 pg/ml in the heterosexuals, 19.0 \pm 7.0 in the homosexuals, and 17.5 \pm 7.5 in the transsexuals.

After administration of conjugated estrogens, serum testosterone and LH levels decreased significantly in all groups ($P < 0.01$; Fig. 3). The fall in serum testosterone was greater among male homosexuals than in male trans-

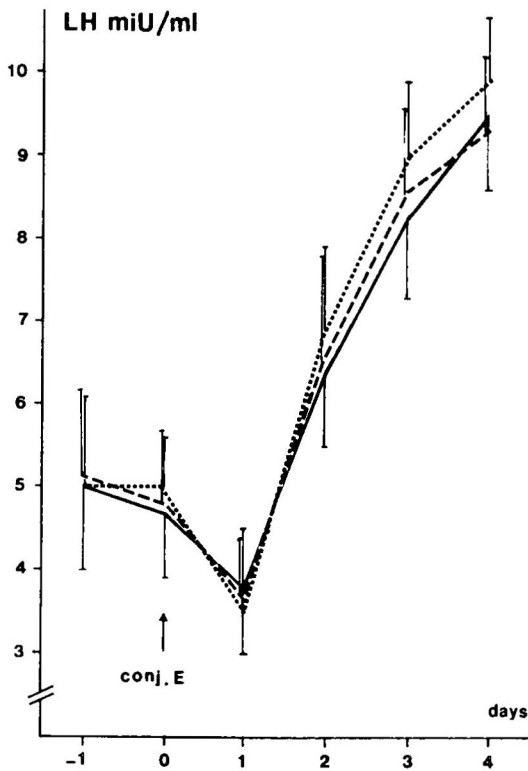


FIG. 1. The response (mean \pm SD) of serum LH to administration of a single dose of conjugated estrogens (conj. E) in heterosexual (—), homosexual (---), and transsexual (· · · · ·) women.

sexuals ($P < 0.05$), but not greater than that of heterosexual men (Fig. 3). The fall in serum LH levels was greater among the transsexuals than the homosexuals ($P < 0.05$), but not greater than that of the heterosexuals (Fig. 3). Differences in the fall in serum LH and testosterone levels between male heterosexuals and male homosexuals were not significant. There was a significant negative correlation between the magnitude of the fall in serum LH levels and that of the fall in serum testosterone levels resulting from administration of conjugated estrogens in all men ($P < 0.05$). On day 4, the serum LH levels of the male homosexuals were significantly higher than those of the male transsexuals ($P < 0.05$), but not higher than those of the male heterosexuals (Fig. 3). At this time, the serum LH levels of the homosexuals, but not those of the transsexuals and heterosexuals, had returned to or were above the values on days -1 and 0 (Fig. 3).

In none of the male homosexuals or transsexuals was the response of LH to LHRH stimulation greater after administration of conjugated estrogens (Fig. 4). Elevated serum LH levels on day 4 after the administration of conjugated estrogen compared to levels on days -1 and 0, which most closely approximate the normal female response to conjugated estrogen, were found in 5 of the 15 heterosexual males and 11 of the 23 homosexuals, but

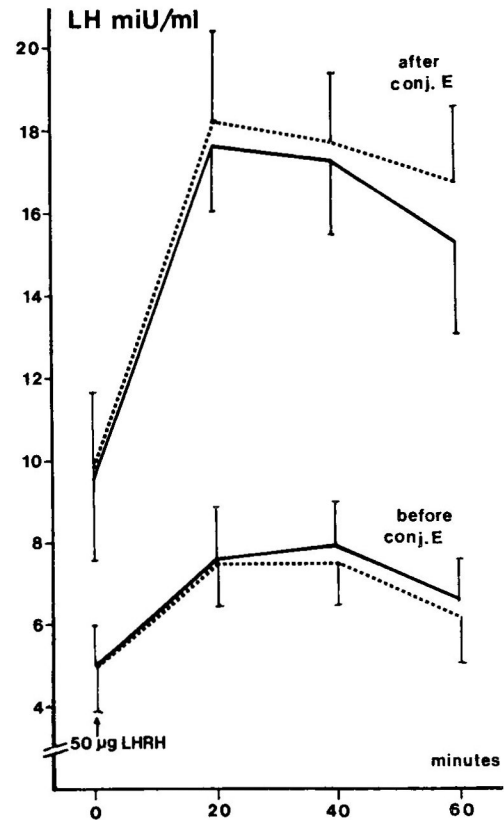


FIG. 2. The response (mean \pm SD) of serum LH to administration of 50 μ g LHRH before and 4 days after administration of conjugated estrogens (conj. E) in heterosexual (—) and transsexual (· · · · ·) women.

in none of the transsexuals.

To explain these different responses, LH and testosterone responses to conjugated estrogens in the subjects who had elevated serum LH levels on day 4 after the administration of conjugated estrogens and in those in whom serum LH had not returned to the basal levels were compared. Hence, the 5 heterosexual males with the female-like response were compared with 6 other members of their group, while 6 of the 11 homosexuals were compared with 7 other members of their group (Figs. 5 and 6). Those subjects whose serum LH levels were higher on day 4 than before conjugated estrogen administration had a significantly greater fall in serum testosterone levels than the subjects whose serum LH levels remained below pretreatment values ($P < 0.05$; Figs. 5 and 6). As these different testosterone responses to conjugated estrogens provided a potential clue to the differences in the response of serum LH levels to conjugated estrogens in the above-mentioned subgroups of homosexuals and heterosexuals, Leydig cell function was assessed with hCG in subgroups of both heterosexual and homosexual men. In those subjects in each group in whom the day 4 serum LH level was at or above baseline values and the fall in serum testosterone levels in re-

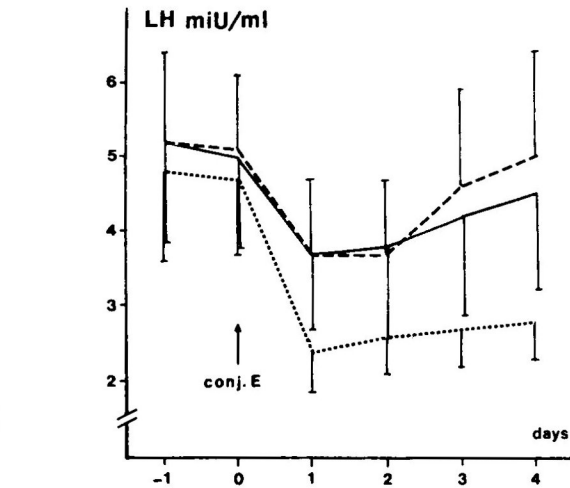
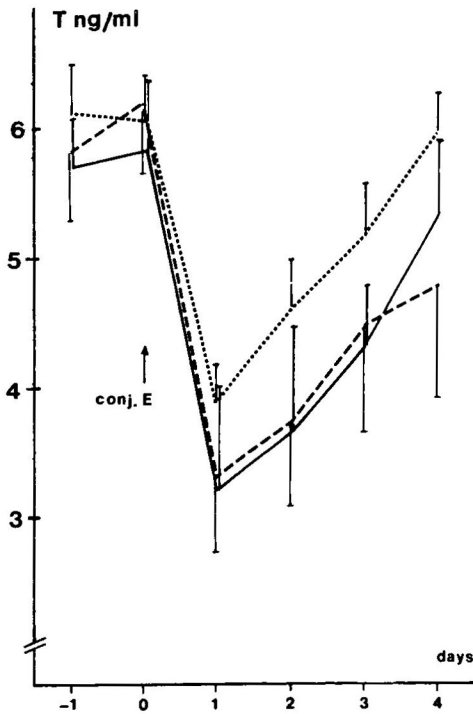


FIG. 3. The response (mean \pm SD) of serum testosterone (T) and LH levels to administration of a single dose of conjugated estrogens (conj. E) in heterosexual (—), homosexual (---), and transsexual (.....) men.

sponse to conjugated estrogens was significantly greater, the serum testosterone response to hCG was significantly less compared to those of others in the same group ($P < 0.05$; Figs. 5 and 6).

An association between 1) the response of serum LH levels to administration of conjugated estrogens, 2) the testosterone response to conjugated estrogens, and 3) the testosterone response to hCG, therefore, was established ($P < 0.05$).

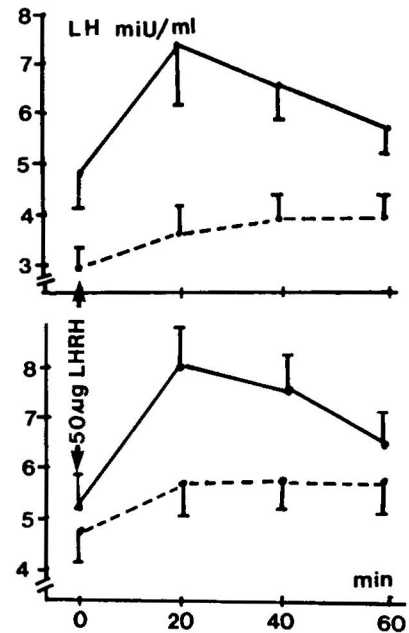


FIG. 4. The response (mean \pm SD) of serum LH to administration of 50 μ g LHRH in transsexual males (upper panel) and homosexual males (lower panel) before (—) and 4 days after (---) administration of conjugated estrogens.

Discussion

This study investigated whether the neuroendocrine response of LH to a single dose of conjugated estrogen could discriminate heterosexuals, homosexuals, and transsexuals of either sex. A difference in response thought to reflect the degree of brain androgenization in homosexuals and transsexuals has been reported (1, 2, 7, 8). I was unable to detect such differences in the estrogen response in the three groups of genetic women studied (heterosexuals, homosexuals, and transsexuals). The response of serum LH levels to estrogen administration in all of these women was clearly greater than that in any of the genetic men studied, while none of the groups of women had any indication of the type of serum LH response to estrogens that was found in the men. These findings confirm previous observations that transsexual and heterosexual women do not differ in their estrogen responses (9, 10) and disagrees with the report of Seyler *et al.* (8), which described an estrogen response of lesser magnitude than that in heterosexual women. The responses of serum LH levels to estrogen administration in the three groups of genetic men were less uniform. The relatively large number of subjects and more rigorous endocrine testing procedures than have hitherto been reported in these types of studies enabled us to analyze the grounds for such variances. Serum LH levels rose highest in those men in whom serum testosterone levels were the most depressed after estrogen administration regardless of sexual orientation (homosexual or hetero-

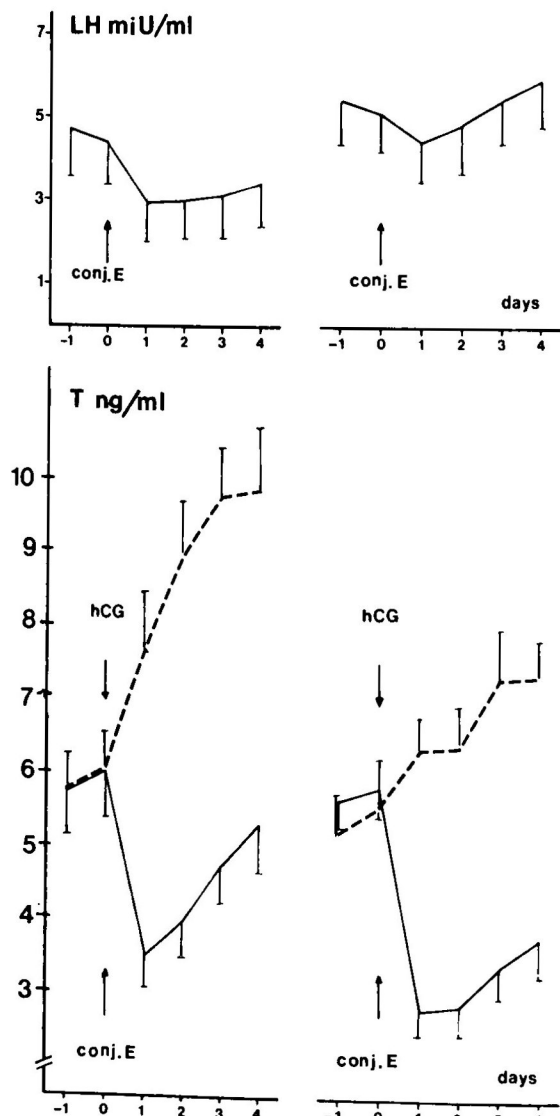


FIG. 5. Comparison of the serum LH and testosterone (T) responses to administration of conjugated estrogens (conj. E) and the testosterone responses to hCG stimulation (mean \pm SD) between six and five heterosexual men in whom, respectively, serum LH levels on day 4 after estrogen administration had not returned to baseline values (left panel) and in whom LH levels on day 4 were equal to or higher than baseline values (right panel).

sexual). The degree of suppression of serum testosterone levels after estrogen administration correlated inversely with the rise of serum testosterone levels after hCG administration, the only test presently available for *in vivo* assessment of Leydig cell steroidogenesis. It is unlikely that the prior estrogen administration would have distorted the testosterone response to hCG administration, since Futterweit *et al.* (19) found an adequate response of testosterone to hCG in male transsexuals receiving chronic estrogen treatment. The results suggest that the quality of testicular steroidogenesis, but not sexual orientation or gender identity, can be regarded as a clue in regard to the estrogen response in the male.

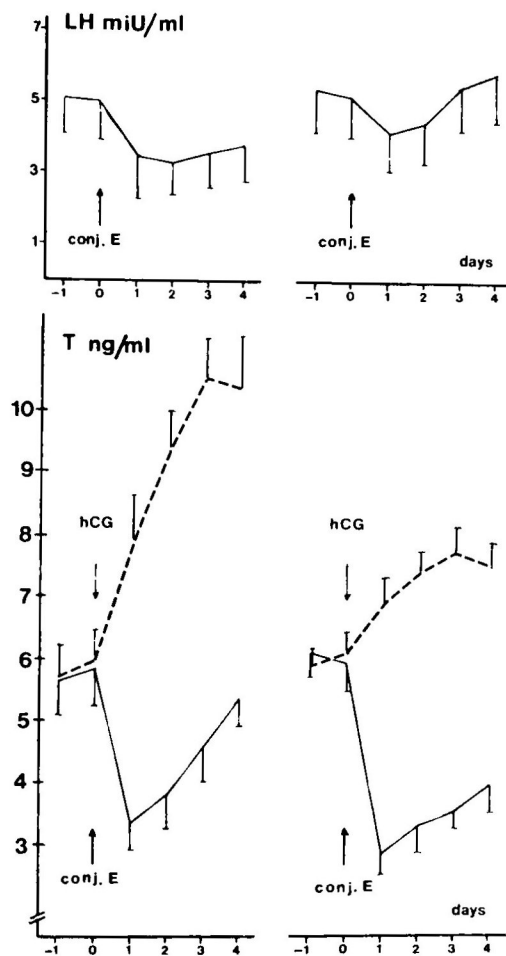


FIG. 6. Comparison of the serum LH and testosterone responses to administration of conjugated estrogens (conj. E) and the testosterone responses to hCG stimulation (mean \pm SD) between seven and six homosexual men in whom, respectively, LH levels had not returned to baseline values on day 4 after estrogen administration (left panel) and in whom LH levels on day 4 were equal to or higher than baseline values (right panel).

It is not immediately clear why some heterosexual men (5 of 15) and some homosexual men (11 of 23) had a greater fall of serum testosterone levels in response to the administration of conjugated estrogens than did others of their respective groups or any of the male transsexuals. Age does have an effect on androgen secretion and the testosterone response to hCG (20). The median age of the homosexual men was highest (33 yr), followed by that of the heterosexual men (29 yr) and the transsexual men (23 yr). A correlation between age and testosterone response to estrogen administration could not, however, be established from our results.

The results do not indicate that the estrogen response can discriminate between heterosexual and homosexual men, as has been reported (1, 2). The findings of Gladue *et al.* (2) that serum testosterone levels fell further in homosexual men after estrogen administration than in heterosexual men after estrogen administration and that

an inverse relationship with serum LH levels could also be detected were reflected in our own results in individual subjects. However, this pattern of response, a sharp fall in serum testosterone levels correlating with a relatively weak response of testosterone to hCG stimulation, was found in a number of heterosexual as well as homosexual men. It is conceivable that more rigorous endocrine testing would have revealed that the response patterns of LH to estrogen administration reported in the studies of Dörner (1) and Gladue *et al.* (2) could be accounted for entirely on the basis of endocrine characteristics of the men without reference to sexual orientation or gender identity. The inclusion in our study of 23 homosexual men as opposed to Gladue's 14 (2) reduces the chance of false positive findings.

A hypothetical correlation between estrogen response and brain androgenization is further controverted by the finding that male transsexuals, who could reasonably be presumed to have the greatest insufficiency of supposed brain androgenization, had estrogen responses that in no way differed from those of the heterosexual men. The responses in these transsexuals did not at all resemble those of any of the women studied.

The female estrogen response is characterized by an increased LH response to LHRH stimulation after estrogen administration (5, 6). All of our genetic women, including the transsexual group, had exactly the same response. This increase in the response of LH to LHRH after estrogen administration, which distinguishes the female and male endocrine systems, was not found in any of our male homosexual and transsexual subjects. Those studies that did find a LH response to estrogen administration in homosexual men that was intermediate between those of heterosexual males and heterosexual females neglected to investigate this endocrine parameter (1, 2). There is now additional evidence that a female-type estrogen response can be evoked in men provided that the appropriate estrogenic environment is present (12-17). These findings should also caution against the premature linking of endocrine findings to such phenomena as homosexuality and transsexualism.

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